Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE

AMERICAN NATURALIST.

Vol. XXXVIII. *March, 1904.* No. 447.

BREEDING HABITS OF CRAYFISH.

E. A. ANDREWS.

Of the breeding habits of the European crayfish much is known, but this is not the case with the American species. The easy assumption that the habits here were essentially identical with those there, has been, possibly, one reason why so little has been put on record regarding our American forms. That there are, however, some considerable differences will appear from the following notes made upon that common species *Cambarus affinis* in 1894 and 1900–1903. Though these observations were made upon individuals kept in confinement in the Biological Laboratory, they may, for the most part, be taken as a guide to what is to be expected from field observations, which still remain much to be desired.

Sex ratio.— The specimens used were taken from the Potomac River in Maryland and when attention was given to the ratio of the sexes it was found that one lot in March 15, 1901, contained 26 females and 14 males while another lot, April 20, 1903, contained 39 females and 14 males. While this shows a marked predominance of females over males at those periods and at that locality it is not general, for a lot of eighty of the same species taken in October, 1903, from a pond in Baltimore, contained 41 females and 39 males. Even where the females are more abundant there is no probability of the eggs going without fertilization since it was found that one male unites with several females.

Time of sexual union.— When specimens were taken in February and put into tanks some unions occurred at once while others were deferred till March. When the sexes were kept separate till March 6 and then put into one tank, unions took place March 6, 7, 8, 9, 10 and 11. After that there were occasional unions, especially of the younger and smaller animals, up to April 2. Besides these observed unions in February, March and April it would seem that there is an Autumnal pairing, in place of or in addition to the Spring pairing. Several small specimens reared from eggs laid in the Spring were found to pair early in October of the same year; while at that same time pairs of larger size were taken in a pond in Baltimore. Moreover specimens from Washington, D. C., in 1894, in November, were seen to pair as soon as they were put into a shallow dish.

Description of sexual union.— The union of the sexes in *C. affinis* was first observed in 1894, and briefly described in the *American Naturalist* in 1895. The same phenomena have been repeatedly witnessed in successive years and there is no doubt that in this species of crayfish the sperm is transferred from the male to an external seminal receptacle upon the female where it is stored up till the time that the eggs are laid. There is no copulation or use of intromittent organs such as takes place in the crabs; nor on the other hand is there any such vague attachment of spermatophores as has been described in the European crayfish, Astacus.

In captivity the union lasts from two to ten hours and either the male or the female may repeat the process with other individuals.

When a female is put into a dish in which a male has been kept till he is accustomed to it he soon seems aware of the presence of the female and does not act as he does when only males are introduced. The preliminary steps toward union are soon taken. The male advances with ready claws and seizes the female, sometimes gently. The female retreats or, when seized, often struggles to escape. Despite these struggles the male holding the female with one of his claws fastened to her antenna or to any projecting part of her head eventually succeeds in turning the female over to lie upon her dorsal surface : if there is no struggle the same result follows more directly and methodically. When the female is turned over the male stands over her ventral surface and later transfers the sperm to the receptacle.

When the first seizure is not effective in leading to a ready turning of the female the male exhibits considerable skill and shows something like intelligence in commanding the new circumstances. Two cases were seen in which the male mounted upon the back of the female and seized her claws as is usual after turning the female over, though in this case turning had not been accomplished. In this unusual position the male attempted to adjust the sperm-transferring appendages to the female and then desisted : then the long antennæ of the male were bent down strongly against the dorsal side of the thorax of the female. In one case the exopodites of the third maxilliped were also used in feeling the female. After getting some information, apparently, by the use of these sense organs, the male proceeded to turn the female over and finally continued a normal sperm transfer. In this turning over the male had to deal with the problem of revolving the female through 180° while under his body and starting with his right claw holding her right claw and his left holding her left so that finally his right held her left and his left her right. In accomplishing this feat the male first removed his left claw from the left claws of the female and with it seized her rostrum and adjacent head region : he then turned the female about 90° so that she lay upon her left side while he stood over her right side. Next the right claw released the female's right claws and seized her left claws. He was now able to turn the female through 90° on to her dorsal surface. Then he transferred his left claw from her rostrum to hold all her three right claws. After that the usual union could take place and ten minutes later sperm was transferred and union continued for some hours.

Usually as soon as the male has thrown the female upon her back he seizes all her clawed legs with his two large claws, holding the three left ones of the female with his one right claw and the three right ones of the female with his left. He then moves forward over the supine female to the position indicated in Fig. 1. From this figure it is evident that the two crayfish are accurately and closely adjusted to one another; not only does the male hold all the claws of the female, but his abdomen is tightly bent around that of the female which is closely coiled up under the male. While all the five right legs of the female may be seen, there appear to be but four legs of the male's set of five left legs. The base only of the male's fifth leg is shown; posterior to this are the peculiar long male pleopods, or appendages of the first abdominal segment and of the second abdominal segment. These four appendages are elevated at an angle of about 45° and point toward the ventral surface of the thorax of the female, forward and downward. A view of the right side of the pair would, however, show the left fifth leg of the male projecting outward and backward between the fourth and fifth legs of that right side.

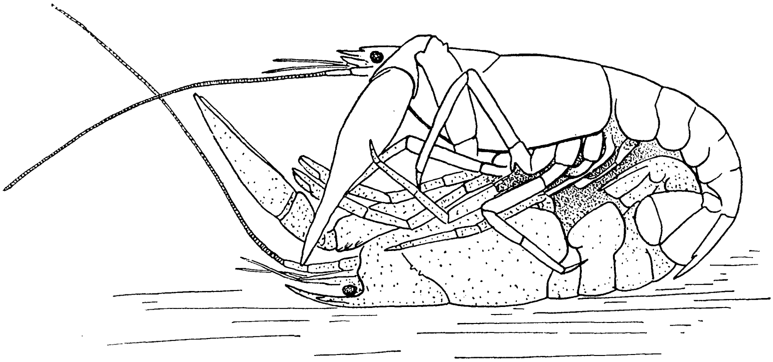

Fig. 1.— Male holding female and with fifth leg supporting abdominal appendages that are about to transfer sperm to the annulus of the female. × $\frac{1}{2}$ diameter.

The explanation of this peculiar arrangement is found in a habit of the male which seems necessary for the accomplishment of sperm transfer and is a very instructive example of mechanical adjustment amongst several rigid calcified organs. After the male has come forward over the supine female there is a period of ten to twenty minutes of apparent inaction before the next move which is as follows: the male rises up away from the female, but still holding all her claws, and deliberately passes one of his fifth legs across under his body so that it projects from the other side. When the male again settles down against the female it is found that the pleopods have the position shown in the figure, whereas before this move they had the normal position, being directed forward, horizontally under the thorax of the male.

It is this forced and remarkable position of the fifth leg which secures the necessary elevation of the male pleopods. These pleopods might be compared to the blade of a pen knife half open and tending to shut up into the handle when pressure is exerted against the tip: and as such a blade might be held in position by a pencil placed across between the blade and the handle and held there, so the pleopods are held in position by the crossed leg which lies anterior to them and between them and the thorax of the male. All these parts are firm and rigid and the pleopods articulate only where they hinge to the abdomen. As the male draws himself down with force against the female the pleopods are so held by the above device, that their tips enter the annulus of the female and the pressure so exerted would tend to shut them down into their resting position, but this is opposed by the fifth leg which blocks the pleopods firmly. That there is force exerted by the tips of the pleopods against the annulus was shown in the case of a female that had been reared from the egg in the Laboratory and when put with a male in April of her second year was covered by a dark blackish deposit after wintering. It was soon found that in union with the male the edges of the opening of the annulus had been scraped clean of the dark deposit and stood out conspicuously against the rest of the dark exo-skeleton.

Though the male always uses one of his fifth legs as an apparently necessary secondary sexual organ it is not always the right or the left; males were seen with right and others with left legs so used. Whether the use of right or left is fixed for each male by circumstances or whether it is a matter of chance or whether inherent in the structure of each male was not determined, but a student who kept many crayfish and sought to determine this point reported to me that one male was seen to use the same leg on one side, in several successive unions with different females.

In one case the fifth leg was seen to project between the third and fourth legs of the opposite side instead of between the fourth and fifth as is the rule. Possibly this may have been in connection with some difference in size between the female and male. Though the two pairing are about the same size there is often considerable difference in length and to secure accurate interadjustment of such rigid bodies with so many protuberances and pairs of appendages is no small problem. To solve it the male at times relaxes his abdomen and moves forward over the female and when finally the tips of the pleopods have been introduced into the annulus the male again envelopes the end of the abdomen of the female and firmly holds it as in the hollow of a hand. The persistent union of the two is made more complete by the use of the hooks found on the legs of the males of this and some other species of Cambarus ; apparently these fasten the male so that the abdominal pressure exerted down the pleopods against the annulus does not react and push the male upward away from the female. In *C. affinis* there is one of these hooks or spines on the third segment of each third walking leg and the male fastens these two hooks into the base of the fourth legs of the female. In the above figures the male has raised the third left leg so that the hook is free and far from its proper socket. When lowered into place the hook depresses the soft membrane that forms the external aspect of the basal segment and thus a temporary socket is formed. The outer ventral edge of this socket is bounded by the stiff calcified ridges that help form the hinge between the first and second segments and it is against this rigid rim that the spine of the male's leg catches. The use of these male hooks as secondary sexual organs is thus established and we may expect to find that in those species of cambarus in which more than one pair of legs are provided with such hooks, that there will be a corresponding increase in complexity of the adjustments of sexual union.

The union of the male and female is now so firm that they cannot be readily separated, and if thrown into actively boiling water the two may be fixed in almost normal positions and serve to make most excellent museum preparations.[1]

Bound together in this way the transfer of sperm from male to female takes place during several hours. Since the crayfish may now be roughly handled or transferred from one dish to another there is little difficulty in observing how the sperm-transfer is effected and a lens may be used without causing the union to cease. The terminal part of the vas deferens of the male, on each side, is found in this period of union to protrude horizontally into the water from the base of each fifth leg as a short, soft, bent tube of translucent appearance. This organ fits exactly into the beginning of the long groove that passes down the first male pleopod, right or left. These pleopods are in fact massive, calcified and rigid tools, each with a deep sinuous groove along it that is seen to lead the sperm from the above ending of the vas deferens down to the tip of the pleopod. The specialized, sharp tips of these organs are inserted into the annulus. The sperm seen to issue from the vas deferens tube glides down the groove of the pleopod to the annulus in the form of long, macaroni-like cords. Microscopic examination of these cords reveals a central axis of real sperm made up almost entirely of the sperm cells and an outer tubular case comparable to soft macaroni. During this transfer of sperm the tip of each pleopod of the second pair is closely applied to the pleopod anterior to it, but no observation was made upon the mode of action of these second pleopods. Each of them has terminal filaments that may well be sensory and also a peculiar, soft, somewhat triangular "spoon" or scoop that fits nicely against the groove of the first pleopod. It would seem that the second pleopods may act to convey sensory impressions and to protect and guide the sperm masses which were found not to go astray but to be in some way retained in the grooves of the first pleopods and forced on into the annulus, probably with the guidance and direction of the second pleopods.

Probably both sides of the body in the male are active at the same time in transferring sperm, but this was not directly observed. During the whole process of union the male is in a state of excitement while the female is quite the reverse, as far as could be judged. The action of the male in turning and adjusting the female is greatly assisted by the state of passivity simulating death that overtakes her soon after being seized by the male. This inertia of the female extends even to the respiratory movements, which seem absent in strong contrast to the condition in the male. The female seems to be dead and the only signs of the continuance of life that were seen were the movements of the eye-stalks in cases where the efforts of the male led to his claws coming against the eyes and, at times, a slight convulsive tremor in the abdomen, possibly connected with sperm transfer. The very small first pair of abdominal appendages which lie often against the annulus and have no probable use unless it be in connection with the phenomena of union, may convey sensory stimuli and occasion the above abdominal contractions. On the other hand the male is in a strongly excited state during the entire period of union: when the struggle and turning have ceased there are still quick vibrations of the anterior maxillipeds and strong currents of water thrown out from the gill chamber as well as the long continued contractions of the limb and abdominal muscles and probably those of the internal male organs. The process terminates when the male moving backward and rising up crosses his fifth leg back again under him into its own side. He then releases the female.

---

[1] The same method may be used to advantage for museum specimens of females "in berry" etc., since the coagulation of the liver, etc., lessens the solubility of substances that ordinarily discolor the alcohol.

The annulus.— Thus the result of union of male and female is the storing up of sperm within the annulus or sperm receptacle of the female. This is in strong contrast to what has been described as taking place in the European crayfish, Astacus, which has no annulus; for French observers state that the male distributes sperm masses, or spermatophores, over large areas of the under side of the female. On the other hand in the American lobster a transfer like that in Cambarus doubtless takes place since Bumpus discovered the sperm-containing receptacle upon the female.

The structure which serves in the lobster to hold sperm is, however, apparently not homologous with the annulus of Cambarus but is different in position and construction. Whether there may not be some kind of receptacle in Astacus, as some appearances there would suggest, something more like the receptacle of the lobster, is a possibility that needs future consideration. It is well known that the annulus differs in many species of Cambarus, and now that we know its use as a sperm receiver we may expect to find some of these specific differences have their uses in the processes of union. The male pleopods are also characteristically and often remarkably different in various species of Cambarus, and these differences may find their explanations in their uses with different styles of annuli.

In *C. affinis* the annulus may be described as a calcified region on the ventral side of the thorax between the sterna of the somites bearing the fourth and the fifth legs. The sternum of the former somite is a wide plate, concave across the middle line and rising up at its edges right and left as two high plates that diverge posteriorly and stand close against the bases of the legs. The sternum of the latter somite is a transverse band enlarging at its ends against the right and left fifth legs and bearing at its middle a transversely elongated rounded elevation. All these parts are hard and calcified. The annulus fills all the space between the above sternal plates and thus lies across the ventral line at the interval between the fourth and the fifth legs. It is close against the sternum anterior to it and may be moved slightly as if hung to it by a stiff hinge.

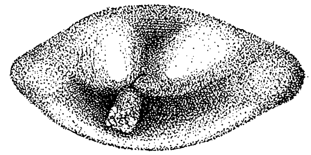

FIG. 2.— Annulus with projecting sperm-plug. × 8 diameters.

In shape the annulus, Fig. 2, is a transverse and elongated ellipse with pointed ends right and left. It is a calcified plate with two elevations, or hills, of varying size and shape near the middle — one right, the other left. Between these elevations is a longitudinal groove and at the bottom of the posterior part of this groove is a narrow chink into which a fine instrument may be forced. This chink opens posteriorly into a deep groove or valley that runs from right to left across the major part of the annulus. This big groove is just posterior to the long axis of the annulus and anteriorly it is overhung, somewhat, by the two hills, while posteriorly it is bounded by an elevated lip or transverse ridge that forms the posterior edge of the annulus and sometimes shows a faint crack in it as if made of right and left halves welded together.

Though the hills are right and left the median slope of one extends across the median line of the body of the crayfish so that the chink between the hills is always asymmetrically placed, sometimes on the right and sometimes on the left. In forty-one females, all but two of which were young, probably but four months old, examined in October, only three cases were found in which the chink was on the left side; in the thirty-eight other females it was upon the right side.

In four still younger females, 32–40 mm. long, the annulus was found less well developed and with but slight transverse groove.

Sections of this organ show that the chink opens into a small rounded pouch or sac which as seen from a dorsal view projects upward as a small, curved ridge. Its walls are stiffly calcified cuticle and no opening could be found excepting the external chink above mentioned. This cavity of the annulus serves as the seminal receptacle, but it is only a small, specialized pit in the external cuticle or exo-skeleton.

After sexual union the female shows a white plug of waxy substance projecting from the chink and filling up the transverse groove at that point as indicated in Fig. 2. This plug is necessarily excentric, generally upon the right side, but in some females upon the left side. It is the surplus sperm envelope or macaroni-like case that came down the pleopod groove and was forced into the chink of the annulus. This plug may remain for weeks, but it disappears some time after the eggs are laid. It may thus be used as evidence of foregoing union for a much greater time than the vaginal plug of some rodents can be. Examining the contents of the cavity of the annulus when the plug is in evidence we find it full of a similar compact paste-like material that may be quite hard and has the form of a tubular sheath around a granular mass that proves to be the peculiar sperm cells of the crayfish. The sperm in this sperm receptacle is in very small amount as compared with the amount produced by one male or with the great mass of eggs to be fertilized, but the actual number of sperm cells is quite large and that they are numerous enough to fertilize all the eggs seems certain though not actually demonstrated. As a fact when females were isolated as soon as union had taken place and kept apart from males till eggs were laid these eggs developed as if fertilized. No sperm was ever seen to be deposited by the male upon other parts of the female than the annulus and if it were not protected as in the annulus it is difficult to see how it could long survive exposure to water which quickly destroys the sperm cells. The laying of eggs, however, may not occur for some weeks after union.

Again, in one case in which the annulus was removed from a female just before laying, the eggs were deposited with normal secretions and habits and though the female was left undisturbed for five days the eggs then appeared shrunken and abnormal, to the naked eye. Twenty-four days later there was only a mass of mouldy dead eggs left on the female. Though there were some cases in which normal females lost most of their eggs by death and fungus yet the above eggs were thought to be all bad from an early date and it is probable they were not fertilized, in the absence of the annulus.

On the whole the evidence seems strongly to favor the view that the sperm received into the annulus in union is the sperm which later fertilizes the eggs. In fact it is possible that sperm taken in antumnal unions may be used for the eggs laid the following spring. Experiments to test this are not yet completed.

Mortality. — The crayfish kept in the laboratory were not fed till after they had laid eggs; then they ate meat, raw and cooked, raw hen's eggs, and pieces of earth worms, as well as Chara and Hydrodictyon. After sexual union many died and it was found that the males died in larger numbers than did the females: thus in one lot seventeen died within two weeks of union in March, and thirteen of those were males and only four females. In several cases the males died within a few hours after union.

Date of laying. — After union there is quite a long period before the eggs are laid: this period is often some weeks. When about 100 males and females were put together March 6–11 the first eggs were laid March 24th, while other females laid from then on to April 15th, though those laid after the first of April were probably unduly retarded by various artificial influences. That March is the normal time of laying for this species and at the Maryland station on the Potomac whence these specimens came is shown by the fact that 39 females taken there April 20th, 1903, all bore eggs in late stages of development that were almost the same as the various stages then present in the eggs laid in March by the previously captured specimens.

Preparation for laying. — The females tend to secrete themselves in dark corners and four or five days before laying they are noticeably excitable, an approaching object causes them to raise their claws and to assume the defensive in a much more active way than was previously the case.

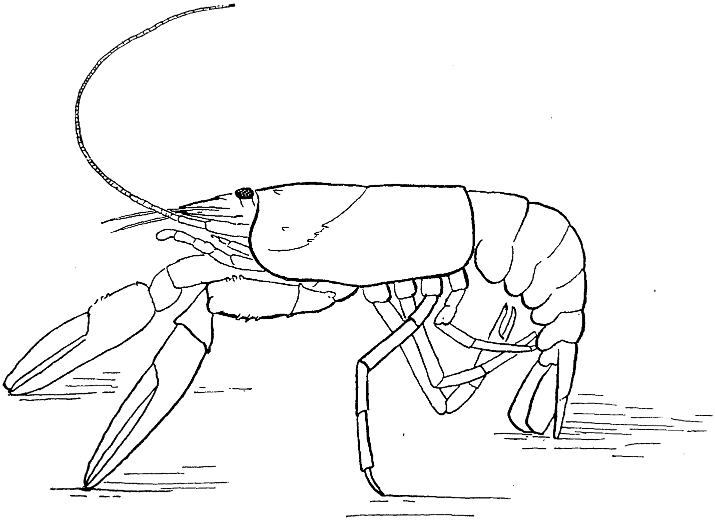

FIG. 3.— Female standing like a tripod and cleaning abdomen with small claws. × $\frac{4}{5}$.

These days are also taken up with great and peculiar activity upon the part of the female resulting in a very thorough cleaning of the ventral side of the abdomen. No matter how darkly discolored the exo-skeleton may have become during the winter it is now made white and clean over the under side which then comes to contrast strongly with the remaining dark exterior.

The process of cleaning was observed in a number of cases and always ran the same course. Though the female is now so alert that it is difficult to catch her in the act of cleaning yet the attitude assumed is most noticeable when seen. As indicated in Fig. 3 the body is raised high up from the usual crouching, crawling position and stands like a tripod supported above the bottom of the aquarium upon the two outspread large claws in front and the oddly down-bent abdomen behind. The other legs aid but little in support of the body and are concerned with the cleaning of the abdomen. The fifth legs, and at times the second and third also, were seen to be thrust back under the abdomen and there carefully and patiently used to remove all the dirt from the entire under surface including the pleopods. Even the numerous long, plumose hairs on the pleopods lose their covering of dark "dirt," and the transformation wrought in the appearance of the whole under side of the abdomen is so great that one would suppose the crayfish had cast its shell and wore an entirely new one.

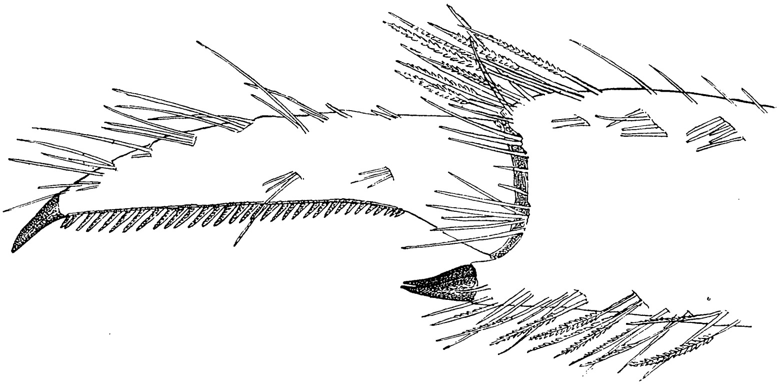

FIG. 4.—Comb and picks on end of fifth leg; used by female to clean abdomen before laying. × 20.

The ends of the fifth legs are shoved against the pleopods and other parts of the abdomen with considerable force but it is only slowly that they accomplish perceptible cleaning. On examining the tips of these legs we find they seem especially well fit for such cleaning work. As indicated in Fig. 4 the terminal segment is like a strong comb, as it has a series of spines along one free edge. At one of the angles of the penultimate segment there is a group of strong picks that look like the horny tips at the ends of legs. In fact these two picks are born upon a truncated process that suggests a homology with the finger of the chelate legs, that process of the penultimate segment which apposes the last segment to form the claw; and we might regard this fifth leg as having lost, or not yet acquired, a perfect claw. The penultimate segment also bears beautifully serrated hairs, as indicated in the figure, recalling the appearance of some setæ of annelids. That these tufts of serrated hairs may serve as scouring brushes seems not improbable. We would then have a double pick to loosen dirt, a stiff comb and a brush with saw-tooth hairs to accomplish the cleaning.

The fourth leg has the same structure and though it was not seen in use it may well take part in cleaning. The second and third legs bear well developed claws and these were seen slowly plucking at the encrusted abdominal surface. As elsewhere shown[1] these claws are well fitted for cleaning as they can pick off objects and are provided with a long row of flat, serrated plates on each limb of the forceps that should serve excellently in cleaning the pleopods.

Time of laying. — In confinement the crayfish deposited their eggs at night time with few exceptions; only one among thirty-two laid eggs in the day time. Yet it was found possible to force the laying of eggs in the day time by keeping the female every night in barely enough running water to moisten the ventral surface of the body and with no opportunity for the normal submergence. When returned to deeper water in the day time eggs were finally laid, at noon, upon the third day of such treatment.

Laying. — The females were easily disturbed and never laid when under observation so that the actual emission of eggs was not seen but the following facts were observed just before and just after the actual laying. A short time before laying the female is sometimes found lying upon her back waving the abdominal appendages back and forth in a rhythm of about

---

[1] *Biol. Bull.* Jan. 1904.

one second. The endopodites of the third maxillipeds and the anterior three pairs of legs are sometimes swung back and forth also. Finally a peculiar secretion is passed forth and the eggs are then laid. This secretion is furnished by the "cement glands" of the under side of the female and needs special notice. One of the things that make much for the clean appearance of the under side of the abdomen when the female is about to lay is the presence of large milk-white areas on the basal parts of both endopodites and exopodites of the sixth pair of abdominal appendages, forming very conspicuous white patches when the tail fan is expanded. The other pairs of pleopods are pretty uniformly milk white but the endopodites are more densely white and the glands in their terminal parts are somewhat segmentally arranged. The sternal plates between all six pairs of pleopods also stand out as milk white areas. Anterior to the abdomen the only milky gland areas are the sternal plate of the last somite, the annulus and the edges of the two flaring sternal plates anterior to the annulus. At other times of year these "cement gland" areas seem inactive or at all events inconspicuous. When a portion of one of the milk areas was removed from the tail fan or from any of the smaller pleopods it furnished, under pressure, a milky material which swelled up in water as a clear jelly containing minute spherules as seen under Zeiss 2. D. and as a somewhat milky glair as seen with the naked eye. When first pressed out from a piece of the glandular area the secretion also contains the minute spherules or droplets.

In one case a female was seen to stand with the body raised high above the bottom of the tank and to wave the pleopods back and forth while they gradually became covered with a clear slime or glair. Forty-five minutes later, at 1.15 p. m. the female was lying upon her back and all the eggs had passed out of the oviduct. The general appearance of this female that had just laid is indicated in Fig. 5. Lying upon the back with the limbs stiffly extended and no motion visible the creature seems dead unless the strongly bent abdomen suggest muscular contraction. Passing forward from the widely expanded tail fan is a faint film of slime or glair that extends to the second legs and neighboring parts. Under this veil a few eggs may be seen not far from the oviduct but the great mass of eggs, several hundred, is concealed by the bent abdomen which encloses them somewhat as a nearly closed hand might a quantity of shot. The actual openings of the oviduct are concealed since the abdomen is bent to its utmost and the tail-fan is carried very far forward over the ventral surface of the thorax. Some slight tremors of globules in the glair near the openings of the oviduct suggested rhythmic contraction of the oviduct, about once a second, even after the eggs had been laid. Gradually the abdomen relaxed somewhat and allowed more of the ventral surface to be seen ; while most of the eggs lay in a mass enveloped by the abdomen, some of them still extended forward on to the thorax as a flat layer. After ten minutes there were some slight movements of the legs and then of the second maxillipeds also. Six minutes later the legs moved more actively and a minute later the crayfish turned itself over on to its ventral side, though this was not easily done in a smooth glass dish.

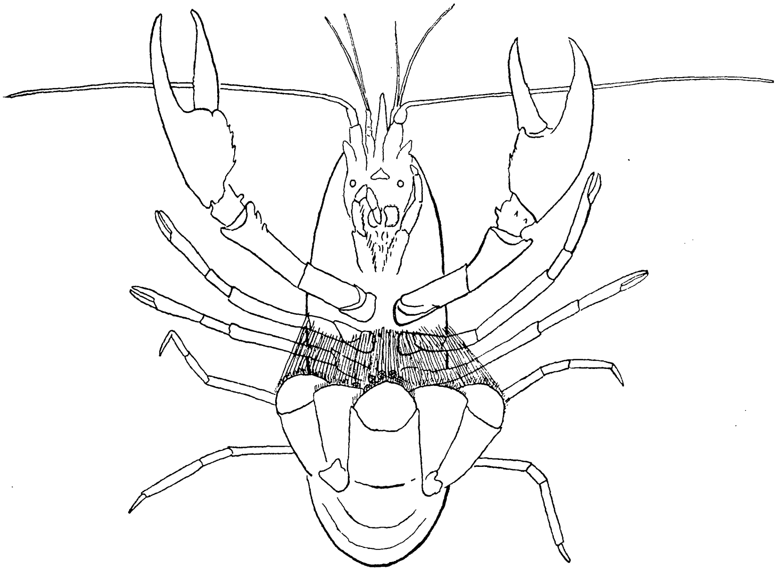

FIG. 5.— Female lying on back with legs held rigid and apron over eggs as they are being laid. × ½.

The eggs must have passed out of the oviducts in a short space of time and have been received into the basket formed by the bent up abdomen, a basket full of glair that would protect the eggs from contact with the water. That this glairy substance is the secretion of milk-white cement gland areas seems certain ; when the material about the eggs is examined under the microscope it contains droplets like those found in the secretion of the cement gland.

The probable mode of laying may be inferred from the above observations and from the following considerations. If we place a female crayfish upon her back and bend the abdomen forward over the thorax as far as possible we can see that if the eggs were forced out by the contractions of the oviducts they would issue in two streams from the mouths of the oviducts which are on the bases of the antepenultimate legs : they would then emerge into a median triangle-like depression formed by the thoracic sterna and this would form an inclined plane down which the eggs would flow into the basket formed by the abdomen, which is on a lower level. When first seen the eggs were very soft, apparently liquid and most easily deformed and indented yet coming rapidly back to a spheroidal shape, owing, partly at least, to the presence of a thin membrane. At first the eggs were not spherical but pear-shaped or elongated ; but when put into hardening liquids they took on a spherical form. When the female is lifted out of the water the soft eggs crowd together and have polyhedral shapes as if liquid, or plastic. In the position assumed by the female it would seem that gravitation acting upon the liquid eggs would bring them into the abdominal basket.

The female that has laid continues to hold the abdomen flexed and the eggs are contained in the basket of glair for some hours. As seen from the side such a female, Fig. 6, seems to have an apron of glair stretching from the second legs and that part of the thorax back to the expanded tail fan and somewhat bellied downward as time goes on. In this condition a remarkable rhythmic habit was observed in several females and regarded as a necessary element in the future success of the eggs. This performance lasts several hours and may be spoken of as : —

Turning.— It consists in a long series of changes in position from the right to the left : the female lies as if dead now on one side and now on the other. After lying a few minutes upon the right the female got back into the ventral position and in a minute or so turned on to the left side, remained there a few minutes and then went back to the ventral position for a minute or two, then to the right side and so on. The striking features in this set of habits are the inert state assumed while lying upon the sides and the great regularity and persistence of the alternating rhythm. The following example will show the details of this process as observed in female XXXII, April 15, 1903, from 1.35–5.50 p. m. As shown in the table this female, as soon as the supine egg-laying position was given up, remained upon her ventral side for three minutes, as in Fig. 6, then turned and lay upon the right side for two minutes, then upon the left side for six minutes, then upon the ventral side for one minute, upon the right side for three minutes and so on.

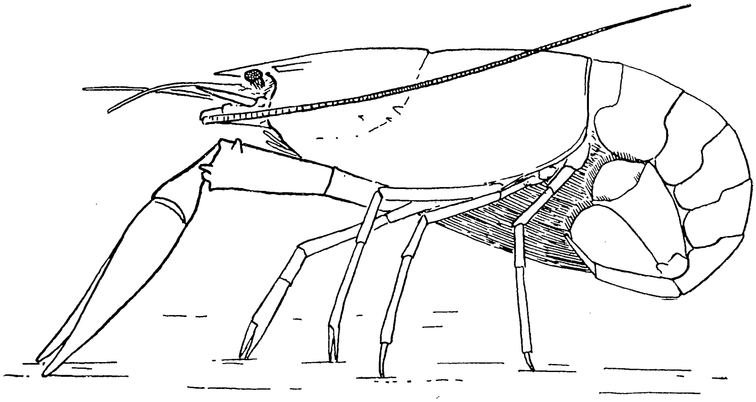

FIG. 6.— Female standing up after laying : apron connecting thorax and bent abdomen. × 4/5.

V3, R2, V0, L6, V1, R3, V1, L4, V2, R2½, V½, L4
V1, R4, V1½, L4½, V½, R3, V½, L4, V1, R2, V3, L6
V2, R3, V1, L4, V1½, R3½, V1, L6, V1½, R3, V2, L4
V½, R6, V0?, L5, V11[×], — — L3, V0, R4, V½, L6½
V3,[†] R4½, V4, L4, V1, R4½, V1, L6½, V2½, R5, V1, L6½
V1½, R6½, V1, L6½, V1, R7½, V1, L5, V2, R7, V½, L5½
V2, R8, V1, L5½, V1, R7½, V1, L4½, V3, R5½, V —

Observations were discontinued with the animal upon the ventral side at 5.50 p. m. In this long series there is only one break in the rhythm at the point marked x when after 51 minutes of turning in which there were ten alternations from right to left; the female then walked about the dish as if seeking some corner more retired than the one she finally returned to and remained in to make the following ten alternations. This walking away from the corner otherwise so closely kept accounts for the long interval of eleven minutes on the ventral side in place of the usual one of only one or two minutes; to it also may be due the break in the close sequence, the turning upon the left side when the right was the one to be expected; unless indeed we assume the time factor to be so dominant as to cause the female to go over to the left side after eleven minutes because that would have been time enough for, say, a ventral rest of 2 minutes, a right rest of 7½ and a left of 1½ which would bring the period around again to a left rest as next in order. At the point marked † the ventral time is abnormally lengthened as it includes the minute and a half spent by the female in struggling to get her ventral position after accidentally rolling over upon her back from the position on the right.

The result attained by this performance would seem to be the proper fastening of the eggs to the pleopods, without which the eggs would not develop. During this process the female stays in one corner of the dish and except when turning from one position to another lies so still that it might be taken for dead; only rarely were any of the claws reached near to the abdomen and there is no evidence of any manipulation to secure the attachment of the eggs within the abdominal basket. It is well known that ultimately each egg is firmly tied to the hairs of the pleopods (a few also to some sternal hairs) by a strong string continuous with a tough membrane that envelops the egg as a sort of second shell. These strings and cases are gradually formed, and from some of the cement gland secretions no doubt. For twelve hours or more after the eggs are laid they may be easily pulled away from the pleopods as they are stuck only by a soft glair, but later this glair hardens and force is needed to break the strings away from the pleopod hairs. In one case when the glair, with many eggs, was taken away from the abdomen soon after laying, new glair was formed within a few minutes. When the egg is freshly laid it is covered by a soft slime that may be squeezed off by rolling it under the cover glass; this material looks like the glandular products above described. The abdominal basket seems full of such material and the eggs sinking down through it are seen in masses on right or left sides according to the position of the female. The pleopods hanging down into this mass are probably pouring out over their hairs more and more of the glandular secretion which will finally harden. By turning the female must let all the eggs fall against now the right and now the left pleopods and again when standing, ventral side down, allow the eggs to dangle down in their coatings of slime till strings would, probably, be pulled out above them attaching each to some part of the pleopod.

As there are four to five hundred eggs laid at one time, in one case 631 eggs by count, it is no light problem to get every one well fastened by its own stalk to the abdominal organs that later serve for their protection and aëration.

Any escape and loss of eggs during the turning action is prevented not only by the abdominal basket of slime with its special apron or surface in contact with the water but by the position assumed by the female; when upon her side the legs under the thorax raise it so that gravity would tend to hold the eggs in the abdominal basket; when upon the ventral side the same is generally true since the abdomen is carried lower and the thorax raised.

After the turning has ceased the glair apron is still in evidence and becoming more conspicuous from discoloration and accumulation of dirt. But after some hours it becomes broken by movements of the pleopods and by straightening of the abdomen and is gradually removed. The claws often have masses of glair upon them and perhaps they assist in removing the superfluous mass. Shreds of glair remain attached to the edges of the tail-fan as late as thirty-six hours after the eggs are laid.

Care of eggs. — Very few eggs fail to become fastened to the pleopods in the above process of turning and henceforth the female bears them so fastened till they hatch. When the eggs were removed and put into dishes of running water they died, except when taken in very late stages with the embryos well formed. The female takes a certain amount of care of the eggs which seems to greatly increase their chances of hatching; as a rule most of the eggs hatch, but in several cases the eggs upon the abdomen became covered by a fungus which bound them all together into one dead, discolored mass. Still the female bore them till long after they should have hatched. That this fungus attacks the eggs in the open was shown by the fact that one of the 39 females taken "in berry" was found to have moulded eggs when received. The female after laying backs into the darkest, most protected corner available and for a long time keeps the abdomen more or less bent down under the eggs which are then protected from dirt; but at times the abdomen is straightened out and the eggs hanging like bunches of grapes from the pleopods are moved back and forth in a manner well calculated to keep them clean and to insure better aëration, Fig. 7.

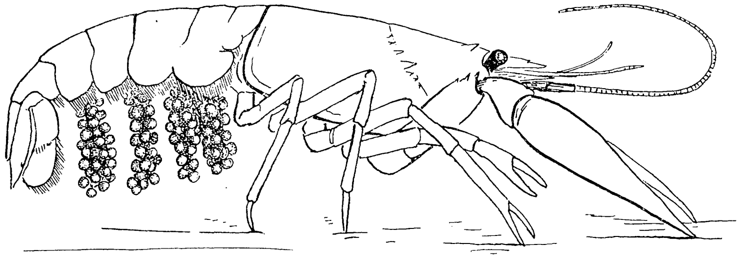

FIG. 7.— Female aërating eggs by raising and straightening abdomen and waving pleopods back and forth. × $\frac{1}{3}$.

Sometimes also the female may be seen reaching back among the eggs with her smaller claws as if to examine or to clean them. Some of the females died before the young hatched out and this was more often the case amongst the females that had laid in the open and not in the laboratory. If the eggs were left upon the dead female they became overgrown by mould and died; but if taken a week before hatching and put into a McDonald fish-jar they hatched successfully in considerable numbers.

Fertilization. — As most of the eggs hatch and as sections of freshly laid eggs show sperm nuclei there seems no doubt that the eggs are fertilized and probably the sperm in the annulus is used for that purpose. But, unfortunately, the mode of fertilization or even its actual occurrence has not been made out. The sperm plug may remain visible for a few days after laying but it then disappears. Its appearance also is changed, its end broken, after laying. Examination of the contents of the annulus after laying showed very few sperms. As the eggs are laid they probably pass over the annulus and a relatively small amount of sperm might fertilize all of them if it came out of the sperm plug at the right time. Before laying the annulus is covered with glair and possibly this may act to bring the sperms out as well as to protect them from the water, which produced marked and apparently destructive changes in the sperms. Some osmotic factor may here be concerned in bringing out the sperm. Other means of getting the sperm out from the waxy tube that we have called the sperm plug and from the interior of the annulus might be: the pressure that the sternal plate between the fifth legs may exert upon the annulus when the legs are forced forward, as sometimes seemed to be the case about the time of laying ; or some activity of the small pleopods of the first abdominal somite. The former action, however, seems only to force the annulus to face more vertically in place of horizontally while the necessity of the first pleopods as instruments in fertilization was disproved by cutting them off from a female about to lay and finding that the eggs underwent normal cleavage as if fertilized.

In support of the view that the sperm issue from the annulus at the time of laying it was found that if the annulus was then removed the female seemed not to be inconvenienced, going through turning movements as usual, but the eggs did not develop. This may, however, have been due to the fact that the female was lifted out of the water, the glair disturbed and thus both eggs, and presumably sperm, exposed to the water, which may have prevented fertilization even if the sperm were upon the eggs.

No sperm were found upon the eggs nor upon the annulus: when sperm are taken from the male or from the annulus they undergo changes of form which might make them less recognizable upon the egg and moreover the egg is 250 times as thick as the sperm and quite opaque.

The whole process of sperm and egg-meeting and union is much in need of elucidation.

Sperm. — While the pairing habits of Cambarus are more complex than those of Astacus the sperm cells are not as complex in form as those described for Astacus by Herrmann. In place of the many radiating arms he figures *C. affinis* has six, as a rule, but sometimes 5, 7 or 8. This is also true of *C. bartoni.* The remarkable bowl shaped vesicle of the crayfish sperm does not have as complex a shape in either of these American forms as it has in Astacus. The diameter of the body is 8 μ while the arms extend out four times that distance on every side so that the entire spread of arms is over 70 μ; but only the refractive vesicle is conspicuous and its longest diameter is less than that of the body.

Period of development, — Attached to the pleopods and presumably fertilized the eggs go through the developmental changes that have been described for Astacus by Reichenbach and others. As these specimens of *C. affinis* were kept in confinement the times of various phenomena could be determined with some precision and in general the eggs of any female develop at about the same rate while the eggs of different females progress differently. Eggs laid near the end of March, 1894, and in 1900, hatched late in May: eggs laid April 11th, 1901, hatched the end of May. In 1903 eggs were laid from March 23d to April 15th; when the eggs were kept in water at $12\frac{1}{2}°$ to $14°$ c some required eight full weeks to hatch; others in a warmer room where the water was not constantly running hatched in six weeks, and some in just five weeks. Most were hatching about May 18th and this was true also of eggs on the females taken April 20th and then in late cleavage or even embryonic stages; so that it seems probable that late in May is a natural time for hatching in the open where these crayfish were taken.

Cleavage. — The nuclear multiplications and migrations of "cleavage" take place but slowly in this heavily yolk laden egg. From sections it seems that the sperm and egg nuclei may be not far from the surface 12 hours after the eggs are laid, while 24 hours after laying the cleavage nuclei are near the centre of the egg and only about ten in number.

There are only a few cleavage nuclei half way out to the surface 48 hours after laying, while 72 hours shows a large number of nuclei near the surface.

In the live eggs some of the cleavage phenomena may be seen with a pocket lens and are much as described by Herrick for *C. immunis*. At about 72 hours there are rounded areas scattered all over the egg, shimmering through the outer part of the egg; each is about 15 μ in diameter and some 30 of them may be seen upon any face of the egg. When such eggs are crushed under the microscope the dense, clear nuclear areas may be found amongst the yolk spherules and also long bundles of fibres connecting the chromatin bodies.

Eggs taken at earlier times from different females differed and also upon the same female. At about 48 hours a few clear areas might be seen in a group deep within the substance of the egg; some eggs showed 5 or 6, some 30–40, some none at all. Later there were similar areas nearer the surface but upon one side of the egg only. Soon these areas spread so that but part of the surface of the eggs failed to show them and finally they are all over the egg.

After the end of the 3d day the increase of these areas by division is plainly indicated by their shape. From 82 hours on to 7 days there are some hundreds of areas each about 150 to 300 μ, some elliptical, others dumb-bell shaped and others in pairs of rounded areas 150 μ in each diameter. There is now a very striking appearance as of light beads floating above the dark background of the egg yolk. As the areas increase in number they become crowded till they finally touch one another and now in profile stand up as hillocks bounded by valleys and projecting into a space of some 100 μ that is formed between the egg and its case.

In late cleavage the color of the egg, which is olive or yellow green at first but varies much in different females, is now more dark sage green and this color with the clear space about the egg gives the entire mass of eggs upon the female a more transparent look that enables one to pick out eggs of this period with ease. Under the lens the eggs present a very attractive appearance as the surface has come to be covered by polygonal cell outlines that form a neat mosaic. These cells at first about 200 μ in diameter and containing nuclear areas 100 μ in diameter become reduced to 50 μ.

The eggs are often much flattened spheroids that readily revolve in their cases so as to keep one face upward. The cells flatten down and the eggs again seem smooth.

After this first week there is a period of apparently little change, nearly a second week in which the eggs turn dark brown and at first were thought to be dead. The surface cells no longer show, while the yolk is arranged in large polygonal areas about 150 μ in diameter and not quite in contact with one another. Reagents, however, show the very many small nuclei of the surface overlying these yolk pyramids.

Embryos. — But before the end of this second week the embryo is outlined somewhat as in Reichenbach's Stages A and B ; that is in gastrula stages.

In the third week Nauplius stages like Stages F and G of Reichenbach were arrived at in from 18 to 28 days in different batches of eggs. Apparently in connection with the very large amount of yolk in these eggs the embryo is small, only 600 μ on an egg 2000 μ.

About the 30th day the embryo had grown to a length of 1000 μ and was about stage H of Reichenbach, that is with some nine pairs of appendages. However one rapidly developing lot of eggs got to that stage in 21 days and hatched out at the same time as a lot of eggs laid two weeks before this rapid lot. Here a difference of temperature was present and may have caused this unusually rapid development. In these exceptional eggs about two days were spent in passing from stages G to H and nine days from H to J. Reichenbach's final stage K was reached 15 days after J and hatching followed in from one to two days.

In *C. affinis* we thus found that cleavage took up the first week, the beginning of an embryo the second week, to progress as far as the Nauplius the third week and more, to enlarge the embryo over one half of the egg a fourth week and more and to perfect the embryo for hatching a fifth and sixth week or more. The whole egg development required from five to eight weeks in different sets of eggs under different temperature.

The heart-beat. — When the embryo has grown so large that it occupies half of the shell and the yolk is correspondingly reduced the beating of the heart is quite conspicuous, more than two weeks before the embryo hatches. Its beat is rapid and may be more than one hundred to the minute and there is the remarkable feature of periodic slowing down and even stoppage. In one embryo the heart beat about 150 times in a minute besides resting some ten seconds so that the rate was very great. There were generally five rests in a minute each of one, two or more seconds each and the intervals between rests were taken up with 26 to 36 beats.

Hatching. — The hatching of the eggs as seen in a watch glass with 2-A. took place as follows. The stiff, transparent case within which the embryo has developed splits open along the side next to the embryo's back as a leather ball might if filled with something that expanded. Before this there were seen some muscular movements within the embryo and now the region still containing yolk material was seen to jerk, the antennæ now and then contracted in jerks and the legs moved slightly. The back of the creature became more and more exposed to the water, I Fig. 8. The legs showed seeming spasms of contraction travelling along them and causing local shortenings. The back of the head-thorax and of the abdomen protruded more and more till only the ends of the body and the limbs remained within the shell, II Fig. 8. The larva thus comes into the world back first. In their development the legs and the abdomen have been formed beneath the thorax and bent forward parallel to it, II Fig. 8; but now the legs are straightened out and raised up more nearly at right angles to the thorax and the abdomen also is moved backward and this aids in pushing the larva out to the position shown in II Fig. 8.

These changes have taken some fifteen minutes and after about five minutes more there is a sudden straightening out of the abdomen which throws the whole creature out into the water as a long straight larva hanging with only the tip of the abdomen left inside the shell, III Fig. 8. The limbs now free in the water kick about and at times the abdomen contracts and since its tip is fast within the shell the result is that the body is brought up near the shell again. The fastening of the abdomen within the shell is brought about by means of a larval skin which is fast to the shell on the one hand and to the anal region of the larva on the other. This skin was seen at the beginning of hatching as a delicate veil over the eyes and doubtless some of the spasmodic contractions seen along the legs were serving to free the limbs from this embryonic skin. The larva thus moults and hatches at the same time and leaves its cast skin within the egg shell to be made use of as a means of keeping the larva from falling away from the mother, for a time. Hanging thus from the egg the young animal soon begins to spasmodically open and shut its large claws and when the body is brought up to the egg case by the contraction

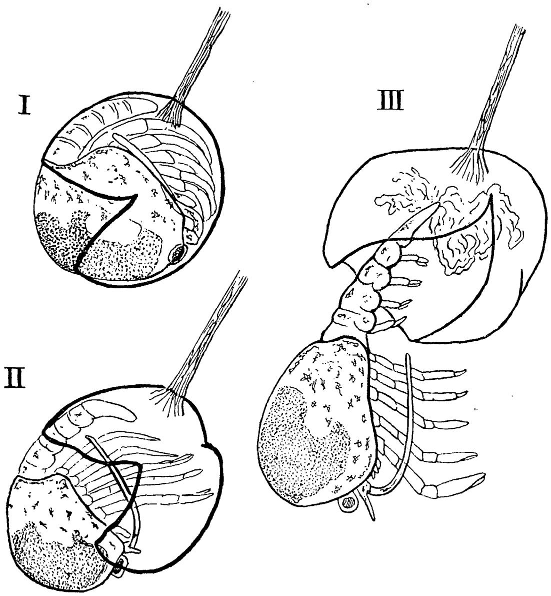

FIG. 8.—Three stages in the hatching of young. In III the larva remains attached by abdomen to cast off skin within egg shell. × 12.

of the abdomen, now and then, the claws finally clasp hold of the stalk of the egg case. As these big claws have the same larval character found in Astacus, that is recurved or hooked tips, they become firmly fixed in the material of the egg-string and apparently the larva could not get free again. The larva is thus made fast fore and aft, its claws are fast to the egg stalk and its anus is fast to the cast skin within the egg case. As the egg cases are still firmly tied to the pleopods by the strong egg stalks the larvæ live still pendent from and for protection dependent upon the mother.

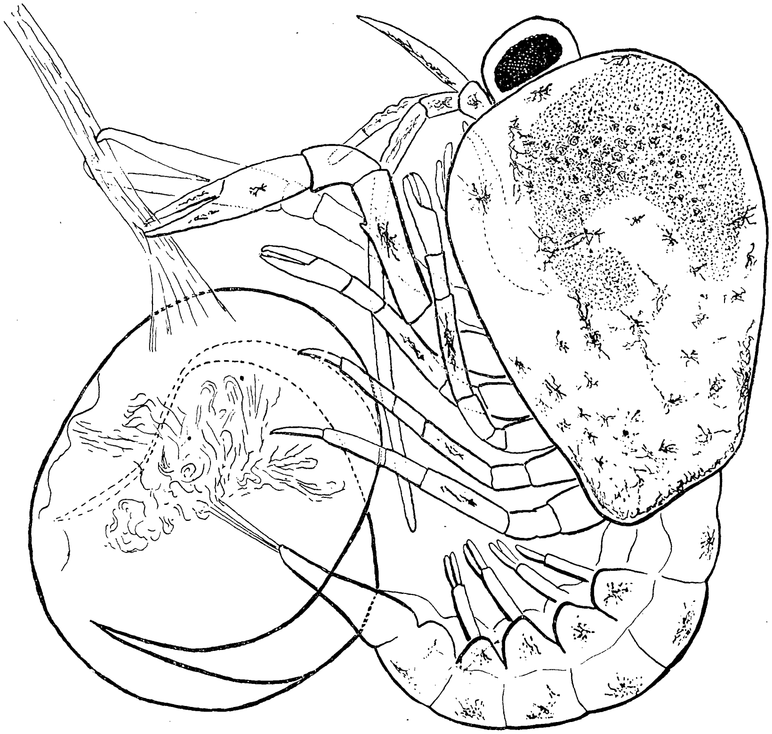

FIG. 9.—Larva 24 hrs. after hatching: claws fastened to stalk of egg shell and abdomen fastened to cast skin inside of egg shell. × 25.

Such a larva 24 hours after hatching is shown in Fig. 9, As in the European crayfish the young is hatched with a swollen globular head-thorax with proportions strikingly unlike those of the adult, but in life the head thorax is not so disproportionally swollen as it becomes in preserved specimens. In this region there is still a large, colored, saddle-shaped dorsal area where the yolk material is present.

All the appendages are present as in the adult except the first and last abdominal appendages so that the abdomen terminates with a very simple broad telson and has no wide tail fan. The eyes are very large. The second antennæ which were packed within the egg shell along the edges of the carapace external to the legs, I, II, III, Fig. 8, soon after hatching come to be between the legs right and left, Fig. 9, and in this dejected position add to the general helpless and incomplete appearance of the larva. The rostrum also aids in this infantile expression of the larva as it is very short and blunt and so bent down between the eyes that it cannot be seen except from a ventral view. Both antennæ and rostrum appear useless, or at least to be poorly placed or perfected to be of such uses as they seem to have in the adult.

This larva is so beautifully translucent that the grossly granular blood corpuscles may be seen projected rapidly along the blood spaces in the thorax, abdomen, legs and antennæ. These corpuscles have a long tail-like process behind and by this they often remain fixed till dragged loose into the current again. Scattered over the thorax and abdomen but not upon the telson are large, branching pigment cells of a crimson color and some are found also upon the antennules and antennæ, upon the fourth segments of the legs and upon the big claws as indicated in Fig. 9.

The creature remains fixed but moves its legs and this causes movements amongst the gills which may be seen through the carapace. There are also at times slight jerking movements of the abdominal appendages.

Respiration seems to be carried on very actively, for the very large scaphognathite may be seen through the carapace, as indicated in the above figure, bailing water out of the gill chamber at the rate of perhaps 180 per minute. The establishment of this rhythmic motion so characteristic of many Crustacea is here a slow process. Before hatching the only rhythmic motion seen was the beat of the heart, but after the creature had emerged into the water this respiratory rhythm was gradually perfected. Two minutes after the larva had first straightened itself out in the water the scaphognathite was seen to swing back and forth but with some stops. Before that at the time of straightening of the body, there were only a few jerky contractions and then, after a minute or more, one or two swings. Five minutes after the body had straightened out in the water the scaphognathite was not in regular swing and even ten minutes was not long enough to acquire an uninterrupted, regular beat.

In some unobserved manner this first or preliminary larva frees its abdomen from the skin within the egg-case, but still remains holding fast by its claws. This larva is about 4 mm. long and lives this life of restricted freedom for only about 48 hours and then moults into a second stage.

Second larval stage.— As the first larva is attached only by its claws we would expect that when it moulted it would become loose and no longer connected with the parent, but such is not the case since it again possesses an anal fastening and later holds by its claws anew. When the moulting takes place the transparent skin of the larva breaks along the back and the back and head of the larva slowly push out through the rent. Then the antennules and antennæ are pulled out of the old skin and for the first time thrust forward into the permanent anterior position instead of being carried backward in the embryonic position retained in the first stage. The legs are pulled out of their old skins and kick about in the water ; then the abdomen is bent free, all but the tip, where the anal region remains fast to that region of the cast skin and does not break loose even when it flaps vigorously. As the shed skin still has its claws fast locked in the egg stalk the larva though it has drawn its hands out of its gloves, as it were, and come out of its old clothes, still remains indirectly fastened to the mother since its telson is fast to its old suit and that is not broken but continuous with the gloves, or claw skins. At first the movements of the legs make the larva seem to be trying to crawl upon the cast skin but after a few minutes the large claws are opened and reaching about take hold of the shed or of the egg case. After many minutes the claws get fastened to the egg stalk again so that the larva is a second time moored fore and aft; by the claws to the egg stalk and by the telson to the old shed which in turn is also fastened by its empty claw skins to the egg stalk. There are thus two real claws and two empty casts of claws fastened to the egg stalk close together. The new claws are still recurved at the tip but it did not appear that the larvæ were always so firmly fixed that they could not get away or at least be rather easily pulled off during the six days that this stage continues.

This moulting was found to take place also when the first larvæ were removed from the mother and kept 24 hours in running water so that the usual protection of the mother is not absolutely necessary for the future development.

The young in this second stage are about 4½ mm. long and have the form represented in Fig. 10. The head thorax is more elongated and crayfish-like in form. The rostrum though bent down between the eyes is much more prominent and when seen from above it has the characteristic lateral spines. The large eyes are prominently stalked. The antennules and antennæ which have some thirty segments, are carried out in front of the animal and moved about as if of use. The abdomen still ends in a bluntly rounded telson with no sixth pleopods as yet free, but these wide lateral appendages may be seen in bags or cases within the lateral part of the larval telson. The whole body is darkened by very numerous golden red pigment cells which are so crowded at the tips of the big claws that these are conspicuously crimson tipped. The yolk-colored area is reduced in extent and dorsally divided into separate right and left areas posterior to end of which is a large green area. On either side of the stomach a blue area adds to the complexity of the above region. The animal still remains somewhat translucent and blood corpuscles can be seen in places. In the antennæ the corpuscles pass from end to end, a distance of about 2 mm. in a few seconds. The legs bear conspicuous hairs not shown in the figure.

When these second larvæ are taken away from the mother they scramble over one another in heaps and do not walk alone, though if forced apart they can stand upon their legs and walk

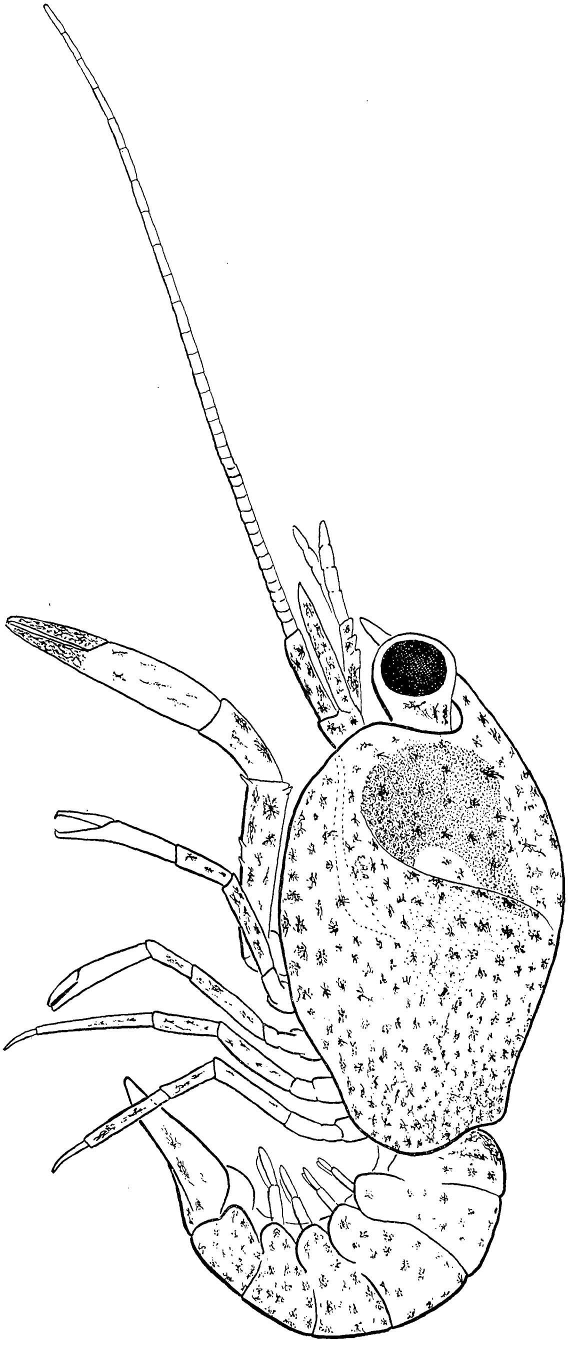

FIG. 10.—Second stage of larva: antennæ now extended: attachment of larva to egg-stalks not shown. × 25.

in a feeble manner. Those that hatched in McDonald jars and moulted to the second stage floated about attached to their sheds and to the moulded egg cases. When placed upon narrow strips of cloth they became entangled so that they could be hung up in the water away from sediment and with some such substitute for the maternal pleopods they could be artificially reared, if need be. Larvæ so suspended probably take food for they were seen to pass out long cylindrical greenish fæcal masses 48 hours after moulting. These larvæ were seen to swim only when thrown into Perenyi's liquid when they darted backward with jerks of the abdomen much as do the adults. They live attached to the mother some six days in this second stage and during this time the yolk areas dwindle away to a large extent while the green areas remained and the blue areas stood out as conspicuous organs in the position of the "crabs eyes" on the sides of the stomach.

Third stage.— As seen in a watch glass the moulting of the larva from the second to the third stage takes but a few minutes. The head and thorax break out first, then the legs are pulled out, the abdomen flaps several times and is freed from the old skin entirely. There is now no anal connection of larva and shed and the free telson is found to have the sixth pleopods fully expanded by its sides so that a very wide tail fan is formed and its efficiency as a swimming organ is enhanced by very long plumose hairs. At length the larva has cut loose from its mother's apron strings. Yet it still remains upon the pleopods of its mother crawling about freely over the old sheds and egg cases. When removed from the mother these young walk about upon their long slender legs much like shrimp and when disturbed spring backward with widely expanded tail fan again suggesting shrimp.

These active third larvæ are 8 mm. long from tip of rostrum to end of telson, while the claws stretch out about 2 mm. in advance of the rostrum and the antennæ are 5 mm. long.

The head-thorax is most decidedly elongated and crayfish like. The eyes remain large and conspicuous and add to the shrimp like appearance. The antennæ still have exceedingly large exopodite scales. The rostrum is now straight out in front, sharply pointed and of the characteristic gothic style of the adult.

The coloring of the larva has been altered by the addition of blue pigment cells over the carapace and legs. The tips of the big claws are no longer crimson but dark red owing to the fineness of the pigment reticulum there. The yolk coloring is gone but the long green hepato-pancreatic areas and the blue "crab's eye" regions show through the carapace.

When these young escape from their connection to the mother they leave behind upon her pleopods the old egg cases and stalks, and the first and second larval skins. These, however, all disappear before very long, but it was not observed whether the female removed them or whether the young may have taken part in tearing them off. The young continue to crawl about upon the pleopods for about a week and before the end of that time the pleopods are cleared of remnants of egg cases.

These free larvæ do not, however, remain continuously upon the mother but gradually make longer and wider excursions into the outer world, returning again and again to the mother. Three hours after moulting some larvæ were found walking about on the bottom of the aquarium and when disturbed they leaped backward several inches with great speed. When the female was lifted up all the young clung fast to the pleopods, but when she was left quiet a few minutes several of the young came off and walk about. The female gave no sign of knowing of the existence of the young but when she walked over a loose larva it turned upon its back, when touched by the pendent mass of young and quickly climbed up amongst the others. But attempts to make the young climb up on to bits of cloth or even shed skins held in a forceps were not successful.

In occasionally swinging the pleopods back and forth the mother was seen to wipe off against the bottom of the tank one of the great number of scrambling young that crowd the pleopods. Such a one at once turned over from its back on to its feet and walked, though it would appear that it probably had had no experience of horizontal surfaces.

The young that walk on the bottom of the aquarium do not stay away long but return to the mother. Thus two days after moulting some 60 young were shaken loose from a female and scattered upon the floor of the aquarium, but in 20 minutes all but 12 were back again upon the mother's pleopods. Four days after moulting the young crawl all over the mother not only over the dorsal side of the abdomen but over the thorax and the head. As many as a hundred may be off at once walking about in the aquarium and climbing up into the chara plants while a little later only a dozen may be away from the mother. When no screen was used and the water ran strongly the young were carried away now and then and in such manner it may be that city reservoirs, such as those in Baltimore, get stocked with *C. affinis*. Eight days after moulting the young had all left the parents though sometimes a few went back to the maternal pleopods during the next few days. Thus by the fifth of June the young are most all self supporting and independent individuals with no other protection than that they find away from the parent.

How far there is any special recognition between the young and the mother was not determined; but when eight young were taken away from the parent and put in a dish with a female from which the annulus had been removed and which had finally lost all her dead eggs without hatching any they climbed up on to her abdomen and claws but soon got off again. The female did not act as did the real mother but moved about restlessly and wiped her mouth parts as if irritated by the young crawling over them. A few hours later there were no young upon her and four of them had disappeared. Again when several females were kept in the same tank there was some evidence that larvæ sometimes crawled up on to the wrong mother. Moreover in 1900 it was noticed that when a dead female was lifted from the water the young that dropped off returned again to crawl upon the dead crayfish and remained even when the parent was far advanced in decay. When the abdomen was broken open the young crawled upon the exposed muscles for a time but later were upon all parts of the abdomen except the broken surfaces.

Later stages.— By keeping the young of a single mother in a tank by themselves some facts were made out as to the subsequent moultings during the rest of May, June and the first half of July. These young had a large aquarium with good supply of mud, Chara, anodons and various plants and insects with running water, sunshine and shallow water for good aëration. The young remained in the third stage for about 18 days and their color gradually changed from red to greenish owing to the great increase in the blue pigment. The legs also developed bands of dark color across them though otherwise almost invisible, yet the big claws stood out as reddish objects with blue at the base. The fourth stage had about the same coloring but being so much larger it was much more easily seen. The rostrum-telson length was 12 mm.: the claws reached out 2 mm. in advance of the rostrum: the antennæ were about 8 mm. long: the thorax was 2½ mm. wide, and the tail-fan expanded 5½ mm. One of these larvæ had short, simple papillæ upon the first abdominal segment to represent the pleopods, apparently of the female. These young were still translucent enough to show the beat of the heart, at a rate of about three beats to the second, and the circulation of blood corpuscles in the antennæ and legs, though the muscles were now much more conspicuous. The effect of the red pigment cells was everywhere toned down by the widely diffused blue cells.

In about 17 days, that is July 1st, many of the young had passed into a fifth stage. The length was now 15 to 18 mm., with the thorax about 3 mm. wide and the tail-fan 8 mm. The color of this fifth stage was white on the ventral side and elsewhere this was overcast by greenish. The legs were now white with dim transverse bands of gray while the large claws had lost the red and showed brilliant blue at the base of both finger and thumb.

The young move about actively, in walking they move the fore pairs of pleopods back and forth very rapidly. They swim readily and are still shrimp like in movement and in appearance, as they have long slender legs, protuberant eyes and wide tail-fans.

Within a week some larvæ were found in a sixth stage with a length of 21 mm., a thorax width of 5 mm., a tail-fan expanse of 11 mm. and antennæ about 19 mm. long.

Some eleven days later a very large larva was found with a length of 29 mm., a width of thorax of 7 mm., claws projecting 8 mm. beyond the rostrum, antennæ 25 mm. long and a tail-fan expanse of 14 mm. This creature was then a crawling crayfish with less tendency to keep high up amongst the water plants: it also was dark greenish, finely speckled, and there was blue on the legs. Little translucency was left and the animal looked much like a diminutive adult. The large claws were still marked with blue and had dark red at their tips.

This specimen was a male with two long, scroll-like pleopods carried forward in the groove under the thorax as in the adult and the following four pairs of pleopods were not modified: thus the male now has but one pair of modified pleopods as is the case in the lobster.

We may summarize the growth and moultings of the young of a single female during two months as follows. The eggs laid the night of March 28th, hatched into a first stage May 18th. With a length of 4 mm. they lived two days and moulted into a second stage. This second stage was 4½ mm., long and lived six days from May 20th. The third stage was 8 mm. long and from May 26th lived 18 days. The fourth stage was 12 mm. long and from June 13th lasted 17 days. The 5th stage was 15–18 mm. long and lived from July 1st five days. The sixth stage was 21 mm. long and from July 6th, lived eleven days. The seventh stage was 29 mm. long, and from July 17th lived an undetermined time as observations were then discontinued. The first and second stages remained attached to the mother for about one week and were not more than 4½ mm. long. The third stage, 8 mm. long, kept on or near the mother for about a week and then lived an independent existence for a week. The following stages were all independent and gradually took on all the adult proportions and colorings.

Growth of young crayfish. — The young were left in a tank with running water, mud, water plants, anodons and frequent feedings with raw hen's egg till October 6th. Then only eight survivors from a hundred or so were found. These were then measured and as seen in Table I they varied greatly in the amounts they had grown, though all were hatched from the eggs of the same mother.

Table I. Measurements of eight brothers and sisters 141 days after hatching.

TABLE I.

	1	2	3	4	5	6	7	8
Length: telson rostrum . . .	62	55	53	49	50	45	43	41
" " chela	75	75	62	65	60	55	41 ?	47
" antenna	37	51	48	46	34	43	—	25
Width: thorax	15	15	14	13	12	11	11	10
" tail-fan	25	23	24	22	25	20	20	19
Sex	♀	♂	♂	♂	♀	♂	♂	♀
Length: anterior male organs .		9	9	9		8	7	
" posterior " " .		10	10	10		9	7	

Ten other young from various females hatched about the same date as the above and measured October 7th gave the following results, Table II.

TABLE II.

	1	2	3	4	5	6	7	8	9	10
Length: telson rostrum	60	59	52	52	45	39	40	33	30	22
" chela-telson	74	65	65	68	55	50	50	41	35	27
" antennæ	45	30	47	46	25+	—	22+	—	20	16
Width: thorax	14	14	13	12	11	10	10	8	8	6
" tail-fan	26	25	23	24	20	20	19	16	15	10
Sex	♂	♀	♂	♂	♂	♂	♂	♂	♂	♀
Length: first male organs . . .	10		9	9	7	6	6	5	4	
" second male organs . .	11		10	10	8	7	7	5	4	

Taking the two tables together the largest crayfish was 62 mm. and the smallest 22 mm. long; the average length was 46 mm. During these two months and three weeks of summer some few seem not to have grown at all, while about one third of them have almost doubled their length. The great difference between the largest and smallest is but imperfectly represented by the above figures: thus the specimens, 10 of II and 1 of I, being in length as 31 to 11 seemed in bulk much more widely apart and in weight they were found to be as 13 to 7. Only 5 of the 18 were females but it is not known when this preponderance of males arose, whether before or after hatching. The males have now two pairs of very conspicuous and well formed sexual pleopods of great size ; the exterior pair are a little less and the posterior pair a little more than $\frac{1}{6}$ the length of the crayfish, but this ratio is the same in the adult. In these young males the antennæ often look longer and more tapering than in the females and that this may now be a secondary sexual character would seem to follow from the measurements. The eight males with perfect antennæ averaged 50.75 mm. in length and their antennæ were 42.875 mm., or 84% of the length of the body. The five females averaged 47 mm. in length and their antennæ 31.4 mm., or hardly 67% of the length of the body.

Under such conditions the young of *C. affinis* may then attain a length of two inches in their first summer. Additional data for knowing their rate of growth were obtained from some young hatched about June 1st, 1901, from eggs laid April 11. These young were kept in a large sink with running water, mud, water plants and anodons which dying from time to time probably gave them food material. The twenty survivors were measured February 11th, 1902, or 8½ months after hatching and the lengths and widths as well as the sex are given in Table III. The sexes could be readily determined as the females had conspicuous annuli and the males well developed sexual pleopods.

TABLE III.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Length	41	38	24	56	32	44	43	39	39	33	33	36	32	34	30	23	30	29	27	32
Width	10	9	6	14	7	11	10	10	9	8	8	9	7½	8	7	5½	7	7	6	7½
Sex	—	♂	♂	♀	♀	♂	♀	♀	♂	♀	♀	♀	♂	♂	♂	♀	♂	♀	♂	♂

Number 4 may have been introduced by mistake : the others range in length from 44 to 23 mm. with an average of 34 mm. None are as long as two inches : one half are about 33 mm. and 80% above 29 mm. The sexes were about equally distributed, 10 males and 9 females.

These young did not attain to as large a size as those in the preceding tables though they were four months older. But this additional time was autumn and winter and it would seem probable that the crayfish of Table III not being as well fed as the others had not grown quite as fast during the summer and had then remained stationary. Though they were kept in a warm room they were in running cold water.

The above twenty crayfish of table III were then kept in the same conditions during the rest of the Winter, Spring, Summer and Autumn and until December 5, 1903. The only two survivors were both females and at that time, being 18 months old, gave the following measurements. One was 70 mm. long and 16 mm. wide: the other 79 mm. long and 20 mm. wide. Since last measured, a space of ten months including a summer, each had *probably* doubled its length.

These two were left in the same surroundings, except when removed for breeding as below described, and measured again July 1st, 1903, when 25 months old. One was 79 mm. long and 20 wide, and the other 76 mm. long and 20 wide. The latter was clean and bright and the former dark and dirty as in winter so that we infer the smaller one had recently moulted and added 6 mm. or about $\frac{1}{12}$ to its length and $\frac{1}{4}$ to its width.

During their third summer these two females were kept with the young of Table I and thus had better food conditions. Only one survived till October 6th and then had a length of 90 mm. and a width of 24 mm., while its tail-fan expanded 43 mm. It had thus gained more than 10 and perhaps as much as 14 mm. in length or added $\frac{1}{8}$ to nearly $\frac{1}{5}$ to its length.

Summarizing the above data we see that the young of *C. affinis* reared in the above conditions of captivity hatch in May from eggs 2 mm. in diameter as larvæ 4 mm. long and there pass through stages of $4\frac{1}{2}$, 8, 12, 15–18, 21 and 29 mm. during the first two months. During the next three summer months they increase to an average length of 40 mm. but may grow as long as 62 mm. During their first winter they may, probably, not grow at all; but in the second summer they may reach a length of 70–80 mm. In the third summer the length may become 90 mm. as indicated by the sole survivor at 28 months.

That this crayfish grows in the open about at the rate above indicated seems not improbable from the measurements made upon 80 specimens of *C. affinis* taken early in October, 1903, from a small pond in Baltimore that had been stocked a few years before. Five of these were 85–100 mm. in length and obviously belonged to a different period of life as there were none between these and 65 mm. lengths. The remaining 75 ranged from 65 to 32 mm. There were only 6 below 40 and 12 above 56; the remaining 57 were from 40 to 56 mm. long. Thus 76% were from 40 to 56 mm. long and we may believe these were hatched that same Spring and were about 4 months old; the same would apply to the few smaller ones. The 12, or 16%, that were above 56 and not above 65 mm. long are too small for young that had passed a second summer and probably were of the same age as the majority. The five largest specimens were too large for second summer crayfish and probably were at the end of their third summer. The absence of any in what we would regard as second year lengths may be perhaps connected with the draining and drying that the pond suffered some winters.

Sexual maturity.— In rearing crayfish it was found that their sexual instincts and organs mature long before the maximum size was attained. Some of the young represented in Tables I and II were found to unite in pairs in October when but 4½ months, or 141 days old. These very small crayfish, two inches long, thus had their sexual instincts developed. Moreover the sperm taken from the male is then, apparently, just like that of the full grown males. The females 1 and 5 of the first table had sperm plugs, though the other females did not. These females were 62 and 50 mm. long. The female No. 2 of the second table was clasped by male No. 3 and subsequently had a sperm plug: this female was 52 mm. long. Whether these females of 50, 52 and 62 mm. in length will lay eggs next spring remains to be seen. The males seen to unite with females were 55 and 59 mm. long.

The two females described in the last section when 70 and 79 mm. long and 22 months old were put with males April 10, 1903. Though they had been alone for some months they at once were united with the males and the annulus of each showed a sperm plug as it did not before. When they were 23 months old they both laid eggs.

Amongst the females that laid eggs in captivity there were both long specimens 120 mm. long and smaller ones down to even 75 mm. From their size we infer these small ones were about to begin their third summer. Thus though the sexual unions take place at the end of the first summer we have, as yet, no evidence of eggs being laid till the beginning of the third summer. However the autumnal union in the first year was found not only in the specimens reared in confinement but among those eighty above mentioned as taken in the open. And here it occurred both in the laboratory and in the pond and not only between the larger, third year sizes but between those of 56 mm. which, probably, were in their first year.

We see then that little specimens of *C. affinis* may have mature sexual instincts and unite in pairs in the autumn when they are but 4 months old and 50–60 mm. long, but as yet it is not known if they lay before they are 23 months old and 75 mm. long.

Finally.— In connection with the development of sexual maturity we may here refer again to the apparent sexual difference in lengths of antennæ noticed in connection with tables I and II. When the above 80 crayfish taken in the Autumn and thought to be most all of the first year, like those of the tables, were measured little difference between the lengths of the male and female antennæ was found. Throwing out the specimens with broken antennæ there were 37 males with an average length of antenna of 42 mm. and 36 females with an average length of antenna of 41 mm. Again the average male antenna was 81.3% the average length of the male body while the same for the female was 78.9%. The differences are so slight that, considering the errors in measurement, there seems here not enough evidence that the females have the shorter antennæ as a class.

BALTIMORE, November 5, 1903.